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REGIONAL BRAIN ACETYLCHOLINE LEVELS IN RATS ACUTELY TREATED WIT--ETC(U)  
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)  A study of brain acetylcholine (ACh) levels was undertaken in an attempt to reconcile discrepancies reported on the effects of ethanol treatment on the cholinergic system. ACh levels were measured in six areas of brains of male Sprague-Dawley rats after a single dose of ethanol or after the induction of ethanol dependence. At various times after treatment the rats were euthanatized by focused microwave irradiation. The excised brains were dissected into the following parts:		

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20. ABSTRACT (continued)

cerebellum, brain stem, hypothalamus, hippocampus, caudate nucleus and cerebral cortex. After an acute dose of ethanol, ACh levels increased in most areas of the brain when blood ethanol concentrations were quite high. As blood ethanol declined, ACh levels decreased to below control values with similar results observed in ethanol-dependent rats still intoxicated. No significant changes in ACh levels were observed in ethanol-dependent rats undergoing a withdrawal syndrome. These data suggest that ethanol treatment exerts multiple effects on the cholinergic system, but they do not as yet support a role of ACh in the expression of the ethanol withdrawal syndrome. The results of this study provide further information on the development and exploration of models for chronic insults to the brain, such as long-term exposure to toxic chemicals and ionizing and nonionizing radiation.

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## INTRODUCTION

Recent evidence suggests an interaction between ethanol and cholinergic systems in the brain. Erickson and Graham<sup>4</sup> have shown that a single dose of ethanol reduces the release of acetylcholine (ACh) in a dose-dependent manner in the cerebral cortex and reticular formation. They also demonstrated that whole brain ACh levels were elevated. On the other hand, Rawat<sup>15</sup> found depressed levels of ACh with a concomitant decrease in ACh turnover. Because of these differences reported in the literature we have reinvestigated the effect of ethanol on brain ACh levels and have extended our studies to include measurements on six areas of the brain and on ethanol-dependent animals.

## METHODS

Male Sprague-Dawley rats (200-300 g) were treated either with a single dose of ethanol (6 g/kg) or rendered ethanol-dependent. Ethanol was administered as a 20 percent (w/v) aqueous solution by means of intragastric intubation. Ethanol dependence was induced with doses of 9-15 g/kg in three to five fractions daily for a period of 4 days according to the procedure of Majchrowicz.<sup>13</sup>

For ACh determinations the animals were euthanatized by focused microwave fixation (Litton Menumaster 70/50, modified by Medical Engineering Consultants, Lexington, Massachusetts; 1.3 kW) using a 3.5-sec exposure time. The brains were excised and dissected into the following parts: cerebellum, brain stem, hypothalamus, hippocampus, caudate nucleus and cerebral cortex. ACh was extracted and quantitated by the radioenzymatic method of Goldberg and McCaman.<sup>5</sup> Blood ethanol levels were determined using the Calbiochem Ethanol Stat-Pack. Means were compared by using Student's "t" test.

## RESULTS

A single dose of ethanol (6 g/kg, p.o.) induced an elevation of ACh levels in most areas of the brain studied 2 hours after administration (Table 1). However, statistically significant changes were observed only in the brain stem and

Table 1. Regional Acetylcholine Levels After Acute and Chronic Ethanol Treatment. Values represent the means  $\pm$  S.E. Numbers in parentheses refer to the number of animals in each group. In acute experiments animals received 6 g/kg, p.o., 2 hours prior to the end of the experiment. Blood ethanol levels in acute animals were  $445 \pm 25$  mg/dl, while in ethanol-dependent animals still intoxicated, they were  $219 \pm 16$  mg/dl.

	ACh levels (nmoles/g $\pm$ S.E.)			
	Controls	Acute	Chronic Intoxicated	Withdrawal Syndrome
Cerebellum	7.6 $\pm$ 0.69(22)	8.4 $\pm$ 0.51(5)	7.2 $\pm$ 1.17(8)	6.3 $\pm$ 0.50(7)
Brain stem	24.2 $\pm$ 1.28(21)	30.9 $\pm$ 1.42(5)*	18.5 $\pm$ 0.95(6)*	27.0 $\pm$ 1.69(6)
Hypothalamus	29.6 $\pm$ 2.26(11)	26.7 $\pm$ 1.36(5)	22.8 $\pm$ 1.85(7)	24.1 $\pm$ 1.03(8)
Hippocampus	24.3 $\pm$ 0.93(19)	27.0 $\pm$ 1.55(5)	17.2 $\pm$ 0.85(5)†	23.0 $\pm$ 1.68(8)
Caudate nucleus	46.0 $\pm$ 1.48(10)	57.0 $\pm$ 2.41(5)*	32.2 $\pm$ 2.36(6)†	50.9 $\pm$ 3.54(8)
Cerebral cortex	19.5 $\pm$ 1.04(17)	22.5 $\pm$ 1.28(5)	17.2 $\pm$ 1.17(6)	19.3 $\pm$ 0.90(7)

\*  $p < 0.05$

†  $p < 0.001$

the caudate nucleus. A study of the time course of this effect in the caudate nucleus revealed a biphasic response to the actions of ethanol (Figure 1). At 2 and 7 hours after ethanol administration, striatal ACh levels were elevated about 25 percent, at times when the blood ethanol concentrations were high. As ethanol was eliminated, ACh levels declined, but became significantly depressed about 20 percent at 18 hours. By 24 hours no ethanol could be detected in the blood and ACh levels had returned to control values. No alterations in ACh levels were observed in the other brain areas beyond 2 hours after treatment with ethanol.

ACh levels were determined in ethanol-dependent rats either while still intoxicated or while undergoing a withdrawal syndrome. At the time of death intoxicated rats exhibited signs of ataxia and had blood ethanol concentrations of



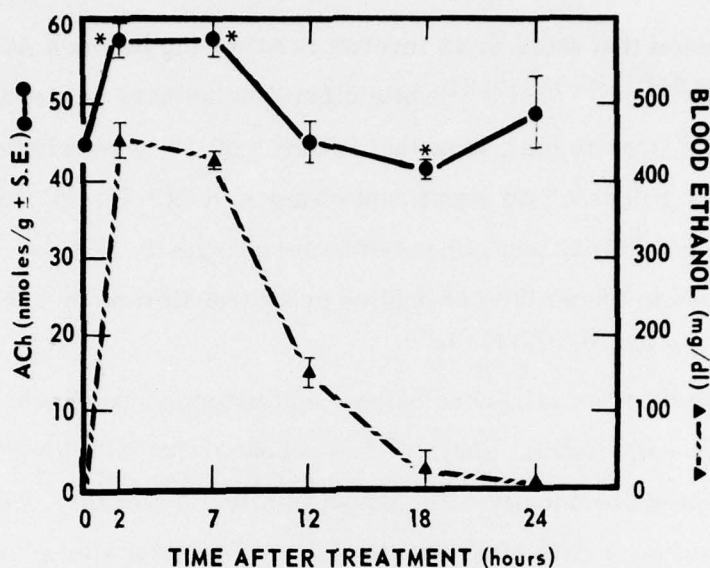


Figure 1. Striatal ACh levels after a single dose of ethanol. Each point represents the mean  $\pm$  S. E. M. of five to ten animals. \* denotes statistical difference from control ( $p < 0.05$ ).

219  $\pm$  16 mg/dl. ACh levels tended to be reduced in all areas of the brain but were statistically significant only in the brain stem, hippocampus and caudate nucleus (Table 1). When ethanol was eliminated from the blood, a fully developed withdrawal syndrome was observed exhibiting signs of tremors, rigidity and convulsions as described previously.<sup>7,13</sup> During this time no alteration in ACh levels could be detected (Table 1).

#### DISCUSSION

Present evidence suggests that the primary effect of ethanol on the cholinergic system is the reduction of ACh release. Studies both *in vitro*<sup>1</sup> and *in vivo*<sup>4</sup> have shown that physiologically compatible concentrations of ethanol significantly depress the rate of ACh release. Studies of ACh release in brains of chronically treated animals have not been reported.

Whether alterations in the rate of release of ACh could be responsible for changes in ACh levels is not entirely clear. The results from several studies

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have suggested that there is an inverse relationship between ACh levels and ACh release<sup>2,12,14,16,17,21</sup> while other studies have not tended to support this concept.<sup>6,18</sup> Our results show that in areas of the brain where ethanol depressed ACh release,<sup>4</sup> no significant changes in ACh levels could be found. An alternative possibility that ethanol-induced changes in ACh levels are a result of alterations in the activity of choline acetyltransferase or acetylcholinesterase has been excluded.<sup>3,10,11,19,20</sup>

A role of ACh in the development and maintenance of ethanol dependence has not been established. Rawat<sup>15</sup> has reported that ACh levels are depressed in mice treated chronically with ethanol in a liquid diet for 4 weeks, returning to control values 4 days after withdrawal. We found a similar reduction in ethanol-dependent rats while they were still intoxicated. But when blood ethanol was eliminated and a withdrawal syndrome was present, no changes were observed. The reason for the discrepancies between the two studies is not readily apparent, but may be related to differences in experimental design or to different reactions of rats and mice to ethanol.<sup>9</sup>

In summary, single doses of ethanol have a biphasic effect on striatal ACh levels. A reduction is observed at low blood ethanol concentrations, while an elevation is found at high concentrations. This is similar to the biphasic effect of ethanol on catecholamine turnover.<sup>8</sup> The reduction in ACh levels observed in ethanol-dependent animals still intoxicated is not present during the subsequent withdrawal syndrome. The role of cholinergic mechanisms in the actions of ethanol on the brain and the development and maintenance of ethanol dependence awaits the results of further research.

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